antibodies generated from Snail protein.

20. (Amended) The method according to claim 18, wherein said step of determining the presence of said diagnostic marker, Snail, is carried out by *in situ* hybridization for a genetic precursor of said diagnostic marker.

21. (Amended) The method according to claim 18, wherein the step of determining the presence of said diagnostic marker, Snail, is carried out by RT-PCR for a genetic precursor of said diagnostic marker, based on extraction of RNA polyA+ of tumour samples and control tissue and the amplification of encoding sequences for said diagnostic marker using appropriate amplimer.

Marked-up copies of amended claims 18-21 are attached.

REMARKS

The Official Action dated July 2, 2002 has been carefully considered. In view of the present amendments and these remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset, it is noted that a shortened statutory response period of three (3) months was set in the July 2, 2002 Official Action. The initial due date for response, therefore,

was October 2, 2002. A petition for a two (2) month extension of the response period is presented with this Amendment and Request for Reconsideration, which is being filed within the two (2) month extension period.

As another preliminary matter, it is noted that claims 1, 6-8 and 13-17 have been withdrawn from consideration in the present application as a result of applicants' election of the subject matter of claims 18-21 in response to the Requirement for Restriction set forth in the Official Action dated February 28, 2002. Applicants reiterate that their election of the subject matter of claims 18-21 in this application is without prejudice to their right to file one or more continuing applications, as provided under 35 U.S.C. §121, on the subject matter of the withdrawn claims.

In the July 2, 2002 Official Action, claims 18-21 have been rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. According to the Examiner, there is insufficient antecedent basis for certain recitations in claims 18-21. Specifically, virtually any term proceeded by the word "the" is deemed objectionable in this regard. The Examiner also characterizes as "not clear" the recitation of "compound" in claim 18 and the words "a generic precursor" in claims 20 and 21. The Examiner also contends in this connection that the meaning of the expression "metastatic capacity", which appears in claim

18, is unclear.

Claims 18-21 have been further rejected under 35 U.S.C. §112, first paragraph as allegedly failing to provide a sufficiently enabling disclosure. It is the Examiner's view, for the reasons stated at pages 3-7 of the July 2, 2002 Official Action, that undue experimentation would be required in order to practice the full scope of the claimed invention.

The foregoing rejections constitute all of the grounds set forth in the July 2, 2002 Official Action for refusing the present application.

In accordance with the present amendment several apparent typographical errors have been corrected at pages 4, 7 and 11 of the specification.

Also, claims 18-21 have been amended to provide proper antecedent basis for the terminology allegedly lacking same, as asserted by the Examiner at pages 2 and 3 of the July 2, 2002 Official Action. Furthermore, the term "compound" in the preamble of claim 18 has been corrected to read "comprising" and the expression "a generic precursor" in claims 20 and 21 has been corrected to read "a genetic precursor", the latter expression referring to the genetic material which encodes the diagnostic marker, Snail, as set forth in the claims. Support for this amendment to claims 20 and 21 is found in original claims 4 and 5.

Applicants have retained the recitation "metastatic capacity" in claim 18 as this recitation is neither vague nor

indefinite. Applicants' position in this regard will be discussed in further detail hereinbelow.

No new matter has been introduced into this application by reason of any of the foregoing claim amendments. Moreover, none of these claim amendments is believed to constitute a surrender of any originally claimed subject matter in order to establish patentability. The effect of these amendments is merely to make express that which was implied in the claims as originally worded.

As a result of these claim amendments, the rejections under 35 U.S.C. §112, second paragraph based on lack of antecedent basis, the misspelling of "comprising" and the lack of clarity of "generic precursor", (which is merely a typographical error) are believed to be overcome.

Accordingly, the only matters remaining to be addressed are the §112, second paragraph rejection of claims 18-21 based on the recitation of "metastatic capacity" and the 35 U.S.C. §112, first paragraph rejection of claims 18-21 based on alleged inadequate enablement. For the reasons set forth below, these two grounds of rejection are respectfully traversed.

A. Claims 18-21 Fully Comply with the Definiteness Requirement of 35 U.S.C. §112, Second Paragraph

The relevant inquiry in determining compliance with the definiteness requirement of 35 U.S.C. §112, second paragraph, is whether the claim in question sets out and circumscribes a particular area with a sufficient degree of precision and

particularity, such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971).

The definiteness of claim language may not be analyzed in the abstract, but must be considered in light of the supporting specification, with the language in question being accorded the broadest reasonable interpretation consistent with its ordinary usage in the art. In re Morris, 44 U.S.P.Q.2d 1023, 1027 (Fed. Cir. 1997). See also Ex parte Cole, 223 U.S.P.Q. 94 (Bd. Apps. 1983) (claims are addressed to the person of average skill in a particular art; compliance with §112 must be adjudged from that perspective, not in a vacuum).

Furthermore, it has long been held that the initial burden of establishing a failure to comply with 35 U.S.C. §112, second paragraph, rests upon the Examiner. In rejecting a claim for alleged indefiniteness, therefore, it is incumbent upon the Examiner to establish that one having ordinary skill in the art would not have been able to ascertain the scope of protection defined by the claim when read in light of the supporting specification. Ex parte Cordova, 10 U.S.P.Q.2d 1949, 1952 (PTO B.P.A.I. 1988).

When the appropriate procedural approach is followed in assessing the claim terminology at issue herein, in accordance with the above-noted authorities, it is beyond question that applicant has satisfied the definiteness requirement of §112, second paragraph, with respect to the subject matter of claims

18-21.

It is clear from the present specification that applicants have discovered a correlation between the occurrence of Snail in epithelial tumors and the invasive and metastatic capacity of such tumors. See, for example, the disclosure at page 7, line 9 through page 8, line 5. This discovery is put to practical application in the method of the present application. As is true of any patentable invention, it is not required that applicants know how or why the occurrence of Snail in epithelial tumors gives rise to invasive and metastatic capacity, but only that it does so. Thus, the various parameters that may be used to characterize "metastatic capacity", as pointed out at pages 2-3 of the July 2, 2002 Official Action, are plainly beside the point.

The identification in a tumor of the capacity for metastasis is a conclusion that results from carrying out the method of claim 18, which clearly sets out the steps of applicants' invention and the objective to be achieved. It is the act of carrying out the recited steps for the purpose of identifying whether a tumor has metastatic (and invasive) capacity that defines the metes and bounds of the claimed invention, and not any particular parameters of metastatic capacity. In other words, anyone who determines the presence of the diagnostic marker, Snail, by performing the several steps of claim 18 has found an indicator of the invasive and metastatic capacity of such tumor, and thus is operating within the metes

and bounds of applicants' invention. It necessarily follows that one of ordinary skill in the art would have no difficulty at all in ascertaining the scope of protection defined by these claims, when read in light of the supporting specification.

In summary, it is applicants' position, with respect to the rejection of claims 18-21 under 35 U.S.C. §112, second paragraph, that anyone of ordinary skill in the art, having applicants' disclosure and claims before him or her, would be apprised to a reasonable degree of certainty as to the exact subject matter encompassed within claims 18-21. Nothing more is required under 35 U.S.C. §112, second paragraph. Cf. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971). Therefore, the §112, second paragraph rejection of claim 18-21 should be withdrawn.

B. Claims 18-21 Fully Comply with the Enablement Requirement of 35 U.S.C. §112, First Paragraph

A rejection under 35 U.S.C. §112, first paragraph, based on alleged inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter as to which the subject matter is not enabling. In re Borkowski, 164 U.S.P.Q. 642 (C.C.P.A. 1970).

Moreover, it is settled law that whenever the adequacy of enablement provided by an applicants' specification is challenged, the PTO has the initial burden of giving reasons, supported by the record as a whole, why the specification is considered inadequate. In re Armbuster, 185 U.S.P.Q. 152 (C.C.P.A. 1975). A properly supported showing that the

disclosure entails undue experimentation is part of the PTO's initial burden under §112, first paragraph. In re Angstadt, 190 U.S.P.Q. 214 (C.C.P.A. 1976).

The primary basis for this rejection appears to be the Examiner's belief that the application does not clearly establish the link between Snail expression and invasive and metastatic capacity of epithelial tumors.

Based on the legend of Fig. 6, the Examiner asserts that:

> Mouse Snail mRNA expression is observed only in the undifferentiated invasive area of chemically induced mouse tumor, not in the undifferentiated invasive area of the tumors induced by PDV or CarB. However, neither the specification nor any art of record teaches a relationship between metastatic capacity of a tumor and expression of Snail."

This assertion is incorrect. The specification teaches in the paragraph bridging pages 7 and 8 that a non-tumoural cell line (MCA3D) and a cell line from a tumour that showed no invasive or metastatic capacity (PDV) did not express Snail, whereas cell lines from tumours with invasive and metastatic capacity (HaCa4 and CarB) expressed Snail. Thus, the specification clearly discloses the existence of the link between expression of Snail on one hand and invasive and metastatic capacity on the other hand.

The Examiner appears to have raised this rejection on the basis of a misinterpretation of the legend of Fig. 6. The Examiner alleges that:

> Snail mRNA expression is observed only in the undifferentiated invasive area of chemically induced mouse tumor, not in the undifferentiated invasive area of the tumors induced by PDV or CarB-induced tumour.

This misinterpretation is two-fold. Firstly, the tumour induced by PDV is well differentiated and non-invasive. See page 8, lines 12-14:

> No expression of Snail was detected in non-invasive, well-differentiated tumours (Fig. 6b and 6d).

As the Examiner will appreciate, Fig. 6b and 6d illustrates the results of in-situ hybridization with Snail in PDV-induced tumours (page 10, lines 6-10). Thus, non-expression of Snail in this tumour does not, as the Examiner appears to believe, represent a false negative, i.e. a tumour with invasive and metastatic capacity that does not express Snail. Rather, it represents a true negative, i.e. a tumour that lacks invasive and metastatic capacity, <u>in keeping with</u> its non-expression of Snail.

Secondly, in contrast to the Examiner's assertion, the undifferentiated invasive areas of the tumour induced by CarB do indeed express Snail. See page 10, lines 12-14:

> Invasive carcinomas do not express E-cadherin (e,g) <u>but do express Snail (f,h)</u>.

Of course, Fig. 6e-h represents the CarB-induced tumour (page 10, lines 6-8). This is not, as the Examiner apparently believes, a false negative, but a true positive.

These comments apply equally to the claim scope rejection set forth at pages 6-7 of the July 2, 2002 Official Action.

To summarise, the involvement of E-cadherin as a tumour suppressor of mouse and human epithelial tumours was well-established before the present invention (see the Prior Art section on pages 1-3 of the specification). The inventors have demonstrated inter alia (1) that Snail is capable of binding to the E-pal element of the mouse E-cadherin promoter and repressing transcription therefrom (see pages 4-6); (2) that Snail in epithelial cells in culture induces phenotypic characteristics associated with invasion and metastasis (see pages 6-7); (3) that in four tumour cell lines tested, Snail expression corresponded precisely with invasive and metastatic capacity: a non-tumoral cell line and a cell line derived from a tumour lacking invasive and metastatic capacity did not express Snail, whereas both cell lines derived from tumours with invasive and metastatic capacity expressed Snail (see pages 7-9, especially the passage cited above); and (4) that Snail expression is inversely correlated with E-cadherin expression (again, see pages 7-9).

The disclosure in the present specification that the expression of Snail inhibits E-cadherin expression and correlates with invasive and metastatic capacity of tumours, in combination with the prior art knowledge of the role of E-cadherin in epithelial tumour suppression, therefore does indeed establish the existence of the link between Snail expression and determination of invasive and metastatic capacity. As required by the claims, following a determination that an epithelial tumour expresses Snail, the teaching of the specification allows

a person skilled in the art reasonably to attribute invasive and metastatic capacity to that tumour. Consequently, it is respectfully submitted that the specification provides enablement for the full scope of claims 18-21.

The enclosed paper, Blanco et al (2002) Oncogene 21:3241-3246, corroborates the link disclosed in the present specification between Snail expression and metastatic capacity of a tumour. The paper reports the analysis of Snail expression in human breast tumours. The Examiner's attention is respectfully directed in particular to the following passages:

> In a series of human breast carcinomas, we have analysed the expression of Snail and that of molecules of the E-cadherin/catenin complexes. We have also correlated these data with the pathological features of the tumours. We show that Snail expression inversely correlates with the grade of differentiation of the tumours and that it is expressed in all the infiltrating ductal carcinomas (IDC) presenting lymph node metastases that were analysed. Considering that Snail is involved in the induction of the invasive and migratory phenotype in epithelial cells, these results indicate that it is also involved in the progression of breast ductal tumours, where it could additionally serve as a marker of the metastatic potential. [abstract, emphasis added]
>
> and
>
> The identification of potentially metastatic tumours is a long-standing goal for oncologists and would be extremely useful in the design of more specific therapies. The loss of E-cadherin expression is considered as a poor prognostic sign and it has been correlated with the transition from adenoma to carcinoma. Since Snail is a direct repressor of *E-cadherin* transcription, the correlation we have found is very likely to

be meaningful. [passage bridging columns 1 and 2 of page 3246, emphasis added]

Based on human tumour biopsies, Blanco et al. (2002) provides peer-reviewed corroboration of the relationship between Snail expression and metastatic potential, to which the claims are directed. This reference, therefore, serves as evidence in support of the preceding arguments.

The Examiner further alleges that the dependent claims lack enablement because of a lack of disclosure (1) that Snail protein is in fact expressed in tumours, because the presence of mRNA does not necessarily correlate with protein expression; (2) of how to make or where to obtain mouse anti-Snail antibodies; (3) of how to make or where to obtain human anti-Snail antibodies and that the identity of human Snail was known at the filing date; and (4) of specific probe sequences for detecting expression of Snail.

These alleged deficiencies will be addressed in turn:

1. The specification refers to Snail as a "transcription factor". The skilled person is well aware, however, that transcription factors are proteins. Thus, it is clear to the skilled person that the mechanism by which Snail represses the E-cadherin promoter will involve Snail in the form of protein, not as e.g. mRNA. The Examiner's objection that mRNA expression does not necessarily correlate with protein expression is theoretically correct, but has no relevance to the present case. Accordingly, the skilled person would appreciate that the presence of Snail protein in a tumour, like the presence of Snail

mRNA, is indicative of invasive and metastatic capacity.

2. As is clear from the specification, the sequence of mouse Snail cDNA is known (see e.g. page 5, lines 11-18). In light of this knowledge, it would be a matter of routine experimentation to express Snail, either receombinantly or in a cell-free system, and to generate antibodies against it, using standard laboratory techniques (indeed, the specification reports the recombinant expression of mouse Snail on page 12, lines 5-12). Detailed protocols for such techniques are available from standard laboratory reference manuals, such as Sambrook et al. (1989) Molecular Cloning: a Laboratory Manual 2nd Edition, Cold Spring Harbor Laboratory Press, NY, Chapters 16 to 18 and Ausubel et al. (1992) Short Protocols in Molecular Biology 2nd Edition, John Wiley & Sons, NY, Chapters 10, 11 and 16.

In light of the public availability of the mouse Snail cDNA sequence, the requirement to generate antibodies against mouse Snail does not represent an undue burden on the skilled person.

3. Submitted herewith is a copy of GenBank accession number AF125377, which discloses the cDNA and amino acid sequences of human Snail. As noted on the copy, the sequences were deposited on 2 February 1999, well before the filing date and priority date of this application. The sequences are also available from other sources, e.g. GenBank accession numbers AF155233 and AF131208, Paznekas et al. (1999) Genomics 62:42-49, and Twigg et al. (1999) Hum. Genet. 105:320-326), all of which

pre-date the filing of this application.

The comments above in section 2. therefore apply with equal effect to this aspect of the enablement rejection also.

4. Again, because the mouse and human cDNA and amino acid sequences for Snail were publicly available as of applicants' filing date, the design and production of probes or primers for detecting the presence of Snail in a biological sample would be a matter of routine experimentation. Again, standard laboratory protocols are available. Ausubel et al. (supra), Chapters 14 and 15.

It is a well-established principle of patent law that a patent specification need not teach, and preferably omits, what is well known in the art. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81 (Fed. Cir. 1986).

Given the description of the invention provided, in the specification, and the state of the art as reflected in the above-cited references, anyone skilled in the art could practice the full scope of the invention, as claimed, without undue experimentation.

In contrast to the clear teaching provided by applicants, the Examiner has failed to provide any probative evidence or sound reasoning, supported by the record or otherwise, that would tend to show the inadequacy of the enablement provided by applicants' specification, as is required by the above-cited case law. Nor has the Examiner identified any

specific subject matter within the scope of applicants' claims for which the present specification is considered non-enabling.

In the absence of adequate evidence or reasoning to support the Examiner's position, the rejection of claims 18-21 based on alleged inadequate enablement cannot be maintained.

In view of the present amendments and the foregoing remarks, it is respectfully requested that the rejections set forth in the July 2, 2002 Official Action be withdrawn and that this application be passed to issue, as such action is earnestly solicited.

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Attachments: Blanco et al., Oncogene, 21:3241-46 (2002)
Gen Bank Accession No. AF 125377

Marked-Up Versions of Replacement Paragraphs

Identification of transcription factors which interact with the E-pal element was undertaken by means of a one-hybrid approximation using the mouse E-pal sequence (-90/-70) oligomerised to direct the expression of the HIS3 gene of *S. cerevisiae* as bait and a cDNA gene library of NIH3T3 fused to the GAL4 [cativation] activation domain as a prey. A total of 130 clones were isolated, capable of interacting with (and directing the transcription of the reporter gene HIS3) the construction containing the native E-pal element; they did not recognise the mutated oligomeric element. This mutated form of the E-pal element contains 2 modified bases (TT instead of GC) which eliminate the E2 box. This mutated form has been described as being responsible for abolishing the repressor effect in the E-cadherin Promoter in mice (Hennig, G., Löwrick, O., Birchmeier, W. & Behrens, J. Mechanisms identified in the transcriptional control of epithelial gene expression. J. Biol. Chem. 271, 595-602 (1996); Faraldo, M.L., Rodrigo, I., Behrens, J., Birchmeier, W & Cano, A. Analysis of the E-cadherin and P-cadherin promoters in murine keratinocyte cell lines from different stages of mouse skin carcinogenesis. Mol. Carcinog. 20, 33-47 (1997).

Analysis of the endogenous expression of Snail by RT-PCR in a panel of cell lines with varying E-cadherin expression demonstrated an inverse correlation between the expression of both molecules and a relationship between the expression of Snail an their invasive and metastatic capacity (Fig. 5). E-cadherin was observed in the epithelial, non-tumoral MCA3D cell line an in the [PCV] PDV tumoral cell line, which in spite of its tumoral origin showed no invasive or metastatic capacity. However, the presence of Snail was not found in any of the cell lines. In contrast, in the tumour cell lines with invasive and metastatic capacity, HaCa4 and CarB, the absence of E-cadherin is associated with the presence of Snail.

The oligonucleotide which contains the sequence of the E-pal element of the mouse E-cadherin promoter (CD-E) (nucleotides -90 to -70) containing targets for the restriction enzymes SalI in 5' and XhoI in 3' was ligated in direct sense for a total of 6 complete repetitions using conventional techniques, isolation in polyacrylamide gels and cloning in pHISi vector (Clontech, Palo Alt, Ca) which contains the reporter gene HIS3 of *S. cerevisia* and replication elements of yeast, bacteria and appropriate selection genes. In this way, the expression of the HIS3 gene remains under the control of the multimerised E-pal element. Correct insertion of the regulatory sequences was verified by PCR, digestion with appropriate restriction enzymes and sequentiation. The bait vector thus generated was denominated pHIS-E6. The same method was used to generate vectors into which a mutant version of the E-pal element was introduced, also ligated 6 times in direct sense, in which the two central oligonucleotides, GC, were replaced by TT. The mutant bait vector generated was denominated pHIS-mE6. The bait vectors pHIS-E6 and pHIS-mE6 were independently integrated in the chromosomal locus URA3 of the yeast strain YM4271 (Clontech, Palo Alto, Ca) by the usual techniques for transformation and selection of stable strains which maintain growth in the presence of 20 mM 3-aminotriazole (3AZT). The strains selected were denominated E-pal HIS3 (native E-pal construct) and mE-pal HIS3 (mutated E-pal construct). The yeast strain E-pal HIS3 was subjected to transformation with a commercial gene library of cDNA fron NIH3T3 cells which contains different inserts of cDNA fused to the GAL4

activation domain in the pACT2 vector (Clontec Palo Alto, Ca), previously amplified to obtain a titre of $3x10^6$ independent clones using conventional techniques. Transformant yeasts were selected for their ability to grow in the absence of Histidine and in the presence of 20 mM 3ATZ, and 300 independent clones were isolated. The plasmids containing the different sequences of cDNA were isolated from the transformant yeasts and were later used to transform *E. coli* (DH5a strain), recovering 221 independent *E. coli* clones, from which the corresponding plasmids were isolated. To eliminate false positives, the 221 plasmids were independently introduced in parallel into the previously generated yeast strains containing the HIS3 gene under the control of the wild E-pal element (E-pal HIS3 strain) or mutated E-pal (mE-pal HIS3 strain), selecting those plasmids which conferred growth in the absence of histidine and leucine and in the presence of 20 mM 3ATZ exclusively in the strain E-pal His3; the total number selected was 130. Inserts of these plasmids were initially analyse using digestion with various restriction enzymes and sequenced in an automatic sequencer. The sequences obtained were analysed in [cDNa] cDNA databanks using the BLAST/FASTA programme. 49% of the clones identified encoded the total or partial mouse Snail cDNA sequence.

Marked-Up Version of Amended Claims

18. (Amended) A method for [the determination of] determining the invasive and metastatic capacity of an epithelial tumour, said method [compound] comprising:

a. obtaining a biological sample from said epithelial tumour;

b. determining [the presence of] whether a diagnostic marker, Snail, is present in said biological sample; and

c. comparing [the presence of] said diagnostic marker determined to be present in said biological sample with its absence in a control sample, the presence of said marker in said biological sample being indicative of the invasive and metastatic capacity of said epithelial tumour.

19. (Amended) The method according to claim 18, wherein [the] said step of determining the presence of said diagnostic marker, Snail, is carried out by using specific anti-Snail antibodies generated from Snail protein.

20. (Amended) The method according to claim 18, wherein [the] said step of determining the presence of said diagnostic marker, Snail, is carried out by *in situ* hybridization for a [generic] genetic precursor of said diagnostic marker.

21. (Amended) The method according to claim 18, wherein the step of determining the presence of said diagnostic marker, Snail, is carried out by RT-PCR for a [generic] genetic precursor of said diagnostic marker, based on extraction of RNA polyA+ of tumour samples and control tissue and the amplification of encoding sequences for said diagnostic marker using appropriate amplimer.